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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
09/621,448	07/21/00	O'DONOHUE M	1533.1010002

EXAMINER	
STEADMAN, D	
ART UNIT	**PAPER NUMBER**
1652	11

HM12/0925
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DATE MAILED: 09/25/01

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

Office Action Summary	Application No.	Applicant(s)	
	09/621,448	O'DONOHUE ET AL.	
	Examiner	Art Unit	
	David J. Steadman	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE ____ MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____ .

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,6-8 and 18-21* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,6-8 and 18-21* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) ____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). ____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other:

DETAILED ACTION

Application Status

Claims 1, 6-8, and 18-21 are pending in the application.

Applicants' amendments to claims 1, 6-8, and 18-21, cancellation of claims 2-5, 9-17, 22, 23, and affirmation of election without traverse of Group I, claims 1-13 and 18-23 in Paper No. 10 are acknowledged.

Applicants' arguments filed in Paper No. 10 have been fully considered and are deemed to be persuasive to overcome some of the rejections previously applied. Rejections and/or objections not reiterated from previous office actions are hereby withdrawn.

The text of those sections of Title 35 U.S. Code not included in the instant action can be found in a prior Office action.

Drawings

1. The drawings submitted with this application have not been reviewed by a draftsperson at this time. Upon allowance of the claims, the draftsperson will perform a review. Direct any inquiries concerning drawing review to the Drawing Review Branch (703) 305-8404.

Claim Rejections - 35 USC § 112

2. The written description rejection of claims 1, 6-8, and 18-21 under 35 U.S.C. 112, first paragraph is maintained. The rejection was fully explained in a previous Office action.

Applicants argue that the amended claims now fully describe the invention such that a skilled artisan would recognize Applicants were in possession of the claimed invention. This

argument is not found persuasive. While the amendments to the claims have further narrowed the scope of the claimed invention, the specification nevertheless fails to sufficiently describe the claimed invention. The claims are drawn to methods of producing L-lysine, L-threonine, and/or L-isoleucine using an altered *C. glutamicum* cell having an increased amount of NADPH, an altered *C. glutamicum* cell having a decreased amount of 6-phosphoglucose isomerase, or an altered *C. glutamicum* cell having a mutant *pgi* gene. However, the specification teaches only a single representative species of either an altered *C. glutamicum* cell having an increased amount of NADPH, an altered *C. glutamicum* cell having a decreased amount of 6-phosphoglucose isomerase, or an altered *C. glutamicum* cell having a mutant *pgi* gene, i.e., a *C. glutamicum* cell with a disrupted *pgi* gene. The specification fails to describe any other representative species by any identifying characteristics or properties other than the functionality of an altered *C. glutamicum* cell having an increased amount of NADPH, a *C. glutamicum* cell with a decreased amount of 6-phosphoglucose isomerase, or a *C. glutamicum* cell with a mutant *pgi* gene compared to an unaltered cell. Given this lack of description of representative species encompassed by the genus of the claims, the specification fails to sufficiently describe the claimed invention in such full, clear, concise, and exact terms that a skilled artisan would recognize that applicants were in possession of the claimed invention.

3. The enablement rejection of claims 1, 6, 7, and 18-20 under 35 U.S.C. 112, first paragraph is maintained. The rejection was fully explained in a previous Office action. Applicants argue that the claims have been amended to narrow their scope and that the specification now enables a skilled artisan to practice the claimed invention without undue experimentation. This argument is not found persuasive. While Applicants' specification is

enabling for a method of producing lysine, threonine, and isoleucine comprising: culturing an altered *C. glutamicum* cell having a disrupted *pgi* gene, the instant specification does not reasonably provide enablement for a method of producing said amino acids comprising: culturing **any** altered *C. glutamicum* cell having an increased amount of NADPH compared to an unaltered cell or culturing an altered *C. glutamicum* cell with **any** mutant *pgi* gene. Factors to be considered in determining whether undue experimentation is required, are summarized in *In re* Wands (858 F.2d 731, 8 USPQ 2nd 1400 (Fed. Cir. 1988)) as follows: (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claim(s). The factors most relevant to the rejection are the quantity of experimentation necessary, the amount of direction or guidance presented, the predictability or unpredictability of the art, and the breadth of the claims. A significant amount of experimentation is necessary to practice the claimed invention as the specification does not provide a rational and predictable scheme for obtaining increased amino acid yields from **any** altered *C. glutamicum* cell having an increased amount of NADPH or a *C. glutamicum* cell having **any** mutant *pgi* gene. Not all *C. glutamicum* cells with increased levels of NADPH or a mutation within the pgi gene will yield an increased amount of an amino acid. Furthermore, the specification has provided no guidance regarding regions of the *pgi* gene structure which may be mutated with an expectation of obtaining a 6-phosphoglucose isomerase with decreased activity. Therefore, it is unpredictable as to which mutations within the pgi gene would decrease 6-phosphoglucose isomerase activity. The scope of the claims is not commensurate with the

enablement provided by the disclosure with regard to the extremely large number of altered *C. glutamicum* cells having an increased amount of NADPH or altered *C. glutamicum* cells having a mutant *pgi* gene. Thus, the specification does not enable one of skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims. Without sufficient guidance, determination of having the desired biological characteristics is unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue. See In re Wands 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988).

Claim Rejections - 35 USC § 103

4. Claims 1, 6, 7, and 18-20 are rejected under 35 U.S.C. 103(a) as being unpatentable over Mascarenhas et al. (Appl Environ Microbiol 57:2995-9) in view of Ishino et al. (J Gen Appl Microbiol 37:157-165), Voet et al. (Biochemistry 2nd Edition, Wiley and Sons, 1995, NY), and Sahm et al. (Ann NY Acad Sci 782:25-39). Claims 1, 6, 7, and 18-20 are drawn to methods of producing L-lysine, L-threonine, or L-isoleucine by culturing an altered *C. glutamicum* cell having an increased amount of NADPH as compared to an unaltered cell or a decreased amount of 6-phosphoglucose isomerase enzyme activity relative to an unaltered cell, wherein L-amino acid yields from the altered cell are greater relative to an unaltered cell, and optionally, wherein said amino acid yields from the altered cell are 1-100 % greater relative to the unaltered cell and optionally, wherein the altered cell has a mutant *pgi* gene.

Mascarenhas et al. suggest that deletion of the *E. coli pgi* gene results in an increased carbon flow through the hexose monophosphate (HMP) shunt resulting in the accelerated

generation of NADPH (p 2998, Discussion, paragraph 3). Mascarenhas et al. do not teach a method of producing L-lysine, L-threonine, or L-isoleucine using an altered *C. glutamicum* cell with an increased amount of NADPH or decreased amount of phosphoglucose isomerase activity relative to an unaltered cell.

Ishino et al. teach that increased carbon flux through the HMP pathway resulted in a greater yield of lysine (p 162, bottom) and that the increased carbon flux through the HMP pathway is due to an increased requirement for NADPH (p 157, abstract).

Voet et al. teach that the biosynthesis of L-lysine, L-threonine, and L-isoleucine requires at least one molecule of NADPH (p 771 and 772).

Sahm et al. teach that wild-type *C. glutamicum* production of L-lysine and L-threonine are feedback inhibited at the level of aspartate kinase (p 56, Figure 1). Sahm et al. further teach threonine production is inhibited by homoserine dehydrogenase and L-isoleucine production is inhibited by threonine dehydratase and acetohydroxy acid synthase (p 33, Figure 5). Sahm et al. teach the creation of *C. glutamicum* mutants with the ability to overproduce L-lysine, L-threonine, and L-isoleucine by mutating and/or overexpressing the above described enzymes, resulting in decreased feedback inhibition (p 37, under *Summary*).

Therefore, based on the combined teachings of Mascarenhas et al., Ishino et al., Voet et al., and Sahm et al. it would have been obvious to one of ordinary skill in the art at the time of the invention for methods of increased production of L-lysine, L-threonine, or L-isoleucine by culturing an altered *C. glutamicum* cell having an increased amount of NADPH compared to an unaltered cell or having a disrupted *pgi* gene. One would have been motivated for a method of producing L-lysine, L-threonine, or L-isoleucine by culturing the overproducing strains of Sahm

et al. with disrupted *pgi* genes primarily because of the teaching of Mascarenhas et al. and additionally because of the teachings of Ishino et al. and Voet et al. as described above. One would have a reasonable expectation of success for methods of increased production of L-lysine, L-threonine, or L-isoleucine by culturing an altered *C. glutamicum* cell having an increased amount of NADPH compared to an unaltered cell or having a disrupted *pgi* gene because of the results of Mascarenhas et al. and Sahm et al. Therefore, claims 1, 6, 7, and 18-20, drawn to methods of increased production of L-lysine, L-threonine, or L-isoleucine by culturing an altered *C. glutamicum* cell having an increased amount of NADPH compared to an unaltered cell or having a disrupted *pgi* gene would have been obvious to one of ordinary skill in the art.

Applicants argue that because Mascarenhas et al. teaches the effects of disrupting the *pgi* gene in *E. coli* and not in *C. glutamicum*, that one would not expect the increase in NADPH in *E. coli* to occur in *C. glutamicum* as well. Applicants further argue that Mascarenhas et al. teach a method of production of tryptophan using *E. coli* with a disrupted *pgi* gene and therefore, an ordinarily skilled artisan would not have a reasonable expectation of producing lysine, threonine, or isoleucine in *C. glutamicum* with a disrupted *pgi* gene as claimed. Applicants' arguments are not found persuasive. One of ordinary skill in the art would recognize that amino acid biosynthetic pathways do not significantly vary between organisms, particularly bacterial strains, and therefore, one of ordinary skill in the art would have a **reasonable** expectation of increased amino acid yields by disruption of the *C. glutamicum pgi* gene. Furthermore based on the teachings of Sahm et al. combined with the references of Macarenhas et al., Ishino et al., and Voet et al., one of ordinary skill in the art would have reasonably expected increased yields of lysine, threonine, and isoleucine.

5. Claims 8 and 21 are rejected under 35 U.S.C. 103(a) as being unpatentable over Mascarenhas et al. in view of Ishino et al., Voet et al., and Sahm et al. as applied to claims 1, 6, 7, and 18-20 above and further in view of Fitzpatrick et al. (IDS reference AS4; Appl Microbiol Biotechnol 42:575-580). Claims 8 and 21 are drawn to methods of producing L-lysine, L-threonine, or L-isoleucine by culturing an altered *C. glutamicum* cell having an increased amount of NADPH compared to an unaltered cell or having an decreased amount of phosphoglucose isomerase activity, wherein the altered cell is produced by subcloning an internal region of a *pgi* gene and inserting the resulting vector into a *C. glutamicum* genome via homologous recombination.

Mascarenhas et al., Ishino et al., Voet et al., and Sahm et al. disclose the teachings described above. The teachings of Mascarenhas et al., Ishino et al., Voet et al., and Sahm et al. do not combine for methods of producing L-lysine, L-threonine, or L-isoleucine by culturing an altered *C. glutamicum* cell having an increased amount of NADPH compared to an unaltered cell or having an decreased amount of phosphoglucose isomerase activity, wherein the altered cell is produced by subcloning an internal region of a *pgi* gene and inserting the resulting vector into a *C. glutamicum* genome via homologous recombination.

Fitzpatrick et al. generally teach a method of inserting an internal fragment of a *C. glutamicum recA* gene into the genome of *C. glutamicum* by homologous recombination for the purpose of gene silencing. The method of Fitzpatrick et al. involves subcloning a fragment of the *recA* gene into a vector (p 578, under *Construction and characterization of recA mutants*) and integrating the *recA* gene fragment into the *C. glutamicum* genome by homologous recombination (p 578, under *Construction and characterization of recA mutants*).

Therefore, it would have been obvious to one of ordinary skill in the art to combine the teachings of Mascarenhas et al., Ishino et al., Voet et al., Sahm et al., and Fitzpatrick et al. for methods of producing L-lysine, L-threonine, or L-isoleucine by culturing an altered *C. glutamicum* cell having an increased amount of NADPH compared to an unaltered cell or having an decreased amount of phosphoglucose isomerase activity, wherein the altered cell is produced by subcloning an internal region of a *pgi* gene and inserting the resulting vector into a *C. glutamicum* genome via homologous recombination. One would have been motivated for methods of producing L-lysine, L-threonine, or L-isoleucine by culturing an altered *C. glutamicum* cell having an increased amount of NADPH compared to an unaltered cell or having an decreased amount of phosphoglucose isomerase activity, wherein the altered cell is produced by subcloning an internal region of a *pgi* gene and inserting the resulting vector into a *C. glutamicum* genome via homologous recombination because of the teachings of Mascarenhas et al., Ishino et al., Voet et al., Sahm et al., and Fitzpatrick et al. as described above. One would have a reasonable expectation of success for methods of producing L-lysine, L-threonine, or L-isoleucine by culturing an altered *C. glutamicum* cell having an increased amount of NADPH compared to an unaltered cell or having an decreased amount of phosphoglucose isomerase activity, wherein the altered cell is produced by subcloning an internal region of a *pgi* gene and inserting the resulting vector into a *C. glutamicum* genome via homologous recombination because of the results of Mascarenhas et al., Sahm et al., and Fitzpatrick et al. Therefore, claims 8 and 21, drawn to methods of producing L-lysine, L-threonine, or L-isoleucine by culturing an altered *C. glutamicum* cell having an increased amount of NADPH compared to an unaltered cell or having an decreased amount of phosphoglucose isomerase activity, wherein the altered cell is

produced by subcloning an internal region of a *pgi* gene and inserting the resulting vector into a *C. glutamicum* genome via homologous recombination would have been obvious to one of ordinary skill in the art.

Applicants argue that due to the deficiencies of Mascarenhas et al. in describing the claimed invention and the lack of disclosure by Fitzpatrick et al. regarding increased amounts of NADPH leading to increased amino acid yields in *C. glutamicum*, these references do not combine to render the claimed invention obvious to an ordinarily skilled artisan. This argument is not found persuasive. As discussed above, an ordinarily skilled artisan would have recognized that amino acid biosynthetic pathways do not significantly vary between bacterial strains, and therefore, one of ordinary skill in the art would have a **reasonable** expectation of increased amino acid yields by disruption of the *C. glutamicum pgi* gene. One of ordinary skill in the art would have applied the method of Fitzpatrick et al. for disrupting the *C. glutamicum recA* gene in order to disrupt the *C. glutamicum pgi* gene. Therefore, based on the combined references of Mascarenhas et al., Ishino et al., Voet et al., Sahm et al., and Fitzpatrick et al., the claimed invention would have been obvious to one of ordinary skill in the art.

Applicants further argue that Fitzpatrick et al. teach away from subcloning genes into *C. glutamicum* by disclosing "'[n]atural recombination systems have hampered some efforts to construct stable vectors in coryneform bacteria by causing plasmid rearrangements and deletions' because coryneform bacteria have high recombinatorial activity and genetic instability" and that disrupting the *C. glutamicum pgi* gene would not be obvious "since the inventors would have had no reasonable chance of success based upon the language in Fitzpatrick et al." (Paper No. 10, p 11). This argument is not found persuasive. At the time of the

invention, one of ordinary skill in the art would have recognized that plasmid instability due to recombination and genetic instability is a common problem with recombinant microorgansims, particularly *C. glutamicum*. However, one of ordinary skill would have recognized that numerous solutions to these problems have been described in the prior art. In fact, Fitzpatrick describe such a solution by stating, "[o]ne way to overcome these limitations is to work in recombinationally deficient hosts" (p 579, Discussion) and describe a method of eliminating recombinatorial activity in *C. glutamicum* (p 576). Futhermore, an ordinarily skilled artisan would have recognized that because of its ability to produce relatively high yields of amino acids, *C. glutamicum* is commonly genetically modified by transformation and genetic insertion of exogenous DNA and/or deletion of endogenous genes, acknowledged by Fitzpatrick et al. in the statement, "[t]he genetic tools and molecular biological techniques for studying gene expression and improving strain productivity in the industrially important coryneform bacteria are now well advanced" (p 579, Discussion). The above cited statement of Fitzpatrick et al. describing plasmid instability of *C. glutamicum* does not teach away or subtract from a reasonable expectation of success for disrupting the *C. glutamicum pgi* gene by homologous recombination. Such statement merely provides justification by Fitzpatrick et al. for specifically disrupting the *C. glutamicum recA* gene, i.e., in order to reduce or eliminate the occurrence of recombination. Threrefore, one of ordinary skill in the art would have had a **reasonable** expectation of success for methods of producing L-lysine, L-threonine, or L-isoleucine by culturing an altered *C. glutamicum* cell having an increased amount of NADPH compared to an unaltered cell or having an decreased amount of phosphoglucose isomerase activity, wherein the

altered cell is produced by subcloning an internal region of a *pgi* gene and inserting the resulting vector into a *C. glutamicum* genome via homologous recombination.

6. No claim is in condition for allowance.

Applicant's amendment of claims 1, 6-8, and 18-21 necessitated the new ground(s) of rejection presented in this office action. Accordingly, **THIS ACTION IS MADE FINAL.** See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to David Steadman, whose telephone number is (703) 308-3934. The examiner can normally be reached Monday-Friday from 8:00 am to 4:30 pm. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ponnathapura Achutamurthy, can be reached at (703) 308-3804. The FAX number for this Art Unit is (703) 308-4242. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Art Unit receptionist whose telephone number is (703) 308-0196.

David J. Steadman, Ph.D.

Rebecca Prouty

REBECCA E. PROUTY
PRIMARY EXAMINER
GROUP ~~1800~~ 1600